developed protocols to use these surface coated quantum dots for intravital imaging in rodents and we are able to successfully acquire in vivo images of the kidney and perform kinetic measurements. Here, we summarize and discuss our results of the PFE property and in vivo imaging and characterization of these quantum dots. This work was supported by NIH DK077051 research award to W. Yu on in vivo kidney imaging.

177-Pos Board B56

Time-lapse Imaging of Individual BK_{Ca} Channels in Live Cells Using Site-specific Labeling of Quantum Dots

Ji-Yeon Kim, **Haedeun Kim**, Sungho Chang, Chul-Seung Park.
GIST, Gwangju, Republic of Korea.

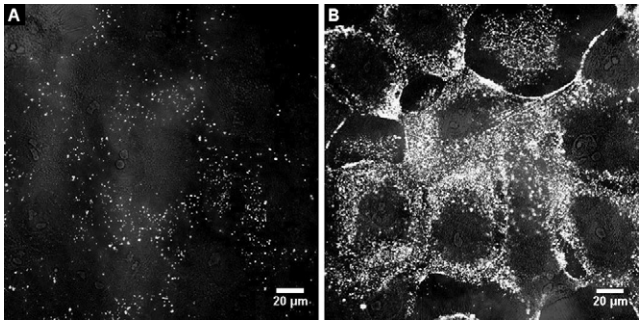
Although the proper localization of specific ion channels at certain regions of cell membrane is essential for their cellular functions, it is a great challenge to visualize and to trace individual channel proteins in live cells. We utilized quantum dots (QDs) to label the large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca} channels) and monitored their movement in real-time. A site-specific biotinylation was achieved by genetically inserting the 'acceptor peptide' sequence at the extracellular N-terminus of the channel and by co-expressing the channel with the *E. coli* biotin-ligase modified to target into endoplasmic reticulum. After brief incubation of streptavidin-conjugated QDs, strong cell surface labeling of QDs was detected in both COS7 cell and cultured hippocampal pyramidal neurons. By tracking the labeled QDs using time-lapse imaging, we were able to monitor single BK_{Ca} channels with high resolution in live cells. In addition, two-color pulse-chase labeling allowed us to observe the channel trafficking to cell surface membrane *de novo* and their redistribution in real-time. Using the time-lapse imaging of QD-labeled channel protein as an assay system, we were able to show the differential roles of cytoskeletons in trafficking and dynamics of BK_{Ca} channels. This new approach can be applied to study the cellular behaviors of ion channels in many different aspects.

178-Pos Board B57

Non-Invasive Pyrenebutyrate-mediated Delivery of Quantum Dots to the Cytosol of Living Cells

Amy E. Jablonski, Christine K. Payne.
Georgia Institute of Technology, Atlanta, GA, USA.

Quantum dots are nanometer-diameter fluorescent probes made of semiconductor materials. Compared to organic fluorophores, quantum dots are highly photostable and very bright making them optimal for live cell imaging. A cationic peptide can be attached to the quantum dot giving an overall positive charge for cell surface binding and subsequent endocytosis. Once endocytosed into the cell, the quantum dots are trapped in vesicles and are unable to access cytosolic components. Pyrenebutyrate, an aromatic, hydrophobic molecule, interacts with the cationic peptide on the quantum dot creating a pyrenebutyrate-quantum dot complex that can bypass the endocytic pathway. The transport of the quantum dot across the plasma membrane is not inhibited at 4°C, lacks colocalization with endocytic markers, and has very little active motion within the cell. This suggests that the mechanism of transport is not endocytosis, but instead direct transport across the plasma membrane. A cell viability assay done with trypan blue determined that incubation with the highest concentration of pyrenebutyrate did not show any harmful effects. By bypassing the endocytic pathway this allows for targeting of cytosolic proteins difficult to label in live cells.



179-Pos Board B58

Characterizing the Architecture of Nicotinic Receptors with Quantum Dot-Based Fluorescence Microscopy

Rigo Pantoja[1], Erik A. Rodriguez[2], Shelly Tzlil[2], Larry Wade[1], Dennis A. Dougherty[2], Henry A. Lester[1].
[1]Division of Biology, Caltech, Pasadena, CA, USA, [2]Division of Chemistry and Chemical Engineering, Caltech, Pasadena, CA, USA.

Ion channel localization and trafficking is important for regulating excitability and synaptic transmission. Quantum dots (Qdots) coated with streptavidin were previously used to label membrane proteins that are recognized by biotinylated antibodies or by biotinylation of an acceptor peptide sequence. We report strategies, suitable for living cells, using strepatavidin-coated Qdots to count and locate extracellular domains of muscle and neuronal nicotinic acetylcholine receptors (nAChRs). Receptors were expressed in *Xenopus* oocytes. (1) The nonsense suppression methodology was used to incorporate the unnatural amino acid biocytin in the muscle nAChR α subunit, in the main immunogenic region, in place of the Asp70 residue. To accomplish this, the *T. thermophila* Gln amber suppressor (TQAS) was chemically aminoacylated with biocytin and co-injected with α70UAG:β:δ:γ mRNA. Functional expression was measured 24 - 48 hours post-injection. The muscle nAChR stoichiometry is $(\alpha)_2(\beta)_1(\delta)_1(\gamma/\varepsilon)_1$; in agreement, two colocalized Qdots were measured by blinking analysis with previously reported algorithms (Pantoja et al, Biophys J in press). (2) The muscle nicotinic receptor was labeled with α-bungarotoxin monoconjugated to biotin (α-Btx-Bio) and subsequently exposed to Qdots; some receptors exhibited the expected two Qdots. (3) The homopentameric α7 nAChR was labeled with α-Btx-Bio and subsequently labeled with Qdots. One to 3 Qdots per α7 receptor were detected, as expected from previous data. In all cases, the robust Qdot fluorescence enabled subunit localization with nanometer accuracy. Strategies (2) and (3) confirm that strategy (1), site-specific unnatural amino acid incorporation combined with Qdot labeling, provides a one-step, specific, efficient labeling approach to investigate composition and real-time trafficking of nicotinic receptors. Grants: NS11756, NS34407, HL79350. Fellowships: Ford and APA-DPN (RP), NSF (EAR).

180-Pos Board B59

HIV-virions Appear To Be Trapped By Human Cervical Mucus

Hacene Boukari, Beda Brichacek, Leonid Margolis, Ralph Nossal.
NIH, Bethesda, MD, USA.

We apply time-resolved fluorescence confocal microscopy and fluorescence correlation spectroscopy to examine the movements of fluorescently-labeled HIV-1 virions (~120 nm) embedded in crude human cervical mucus. Particle-tracking analysis indicates that the motion of most virions is decreased 200-fold compared to that in water and is not driven by typical diffusion. Rather, the time-dependence of their ensemble-averaged mean-square displacements is proportional to $\tau^{\alpha} + v^2\tau^2$, describing a combination of anomalous diffusion at short time scales ($\alpha \sim 0.3$) and flow-like behavior at longer times, τ being the lag time. We attribute the flow-like behavior to slowly-relaxing mucus matrix that follows mechanical perturbations such as stretching and twisting of the sample. Further analysis of the tracks and displacements of individual virions indicates differences in the local movements among the virions, including constrained motion and infrequent jumps, perhaps due to abrupt changes in matrix structure. We surmise that these differences are related not only to possible variations in the local microenvironments experienced by each individual virion but, perhaps, also to variations in the surface structure of the virions themselves. Possible changes in the microenvironments due to slow structural changes may provide a means for some virions to move and reach the port of entry, the underlying cervical mucosa.

Molecular Mechanics & Force Spectroscopy

181-Pos Board B60

Nucleosome Stacking Defines The Structural And Mechanical Properites Of Chromatin Fibers

Fan-Tso Chien[1], Maarten Kruithof[1], Andrew Routh[2], Daniela Rhodes[2], John van Noort[1].
[1]Leiden Institute of Physics, Leiden, Netherlands, [2]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.

In eukaryotic cells, genomic DNA, and core histones form dense 30 nm chromatin fibers. This compaction is driven by stacking of nucleosomes and has been implicated to regulate gene expression. We investigated the mechanical properties of reconstituted chromatin fibers containing 25 repeats of the 601 nucleosome-positioning element. The force-extension curves of these chromatin fibers were measured with magnetic tweezers. The fibers are well characterized by three springs in series: a worm like chain (WLC) of the flanking DNA, a Hookian spring of the 30 nm fiber, and a WLC of the ruptured fiber. Using this analysis we unambiguously demonstrated that nucleosome stacking drives a fiber with 197 bp repeat length into a solenoid helix. The model quantifies force dependent and Mg^{2+} dependent nucleosome unstacking, resolves the structural heterogeneity of 30 nm fiber folding, and reveals fivefold higher nucleosome-nucleosome interaction energy than reported before (Cui and Bustamante, 2000). This provides a complete structural and mechanical description of the high order folding of chromatin fibers.